Engineering Oxidoreductases: Utilization of an unnatural amino acid to create artificial hydrogenases

Final technical report

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0. Abstract:

This report describes progress towards creating peptide-based artificial hydrogenases for interfacing with electrocatalytic applications. The goal of such catalysts is to create hydrogen production or oxidation systems. Basic research in two areas has been pursued. First, artificial amino acids have been developed to tether diiron carbonyl complexes to designed peptides. Dithiol and phosphine artificial amino acids have been generated by covalent modification of a lysine residue. Additionally, an FMOC-protected phosphine-serine derivative has been prepared that can be used in solid phase peptide synthesis. These methods have been used to incorporate metallocenters into water-exposed locations on α-helical and β-sheet peptides, but the catalytic properties were not modified from the small molecule analogues. In a second avenue of research, two new methods for immobilization of protein electrocatalysts at transparent surfaces have been developed. Proteins can be adsorbed to thin (10 nm) gold modified with an alkyl thiol layer, and the resulting submonolayer protein assembly investigated via both UV-vis spectroscopy and electrochemistry. Functional covalent modification of a silane layer on indium tin oxide could also be used as a biocompatible surface for adsorption of proteins that in previous works were denatured by ITO.

1. Objectives:

The objective of this project was to use artificial amino acids to develop designed metallopeptides with active centers related to [FeFe]-hydrogenases, the biological catalysts for the reversible oxidation/production of hydrogen.

Although a considerable number of organometallic small molecule mimics of [FeFe]-hydrogenases have been reported, the exquisite functionality of the enzymes has yet to be replicated in a model system. The hypothesis underlying this project is that a peptide can serve as a useful scaffold to tune metallocenters to achieve useful catalysis. In the long term, such artificial hydrogenases will be interfaced with electrodes to produce electrocatalytic systems for hydrogen production/ utilization. Thus research has been pursued in two parallel directions. First, new strategies have been developed to covalently coordinate metallocenters related to [FeFe]-hydrogenases to designed peptides/ proteins. Second, new approaches for interfacing peptides/proteins with electrodes have been developed. The results from both of these avenues will be described below.

2. Accomplishments/ New Findings:

Artificial Amino Acids

Understanding the chemical principles of hydrogen production/activation at non-precious metal active sites remains an important basic challenge with technological implications for sustainable energy production. The coordination environment of a protein can be difficult to recapitulate in a small ligand, and, in the course of this project, we have pioneered the construction of peptide-based artificial hydrogenases. As shown in Scheme 1, we developed a general method to modify a peptide still attached to its resin support to create an artificial

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14. ABSTRACT

This report describes progress towards creating peptide-based artificial hydrogenases for interfacing with electrocatalytic applications. The goal of such catalysts is to create hydrogen production or oxidation systems. Basic research in two areas has been pursued. First, artificial amino acids have been developed to tether diiron carbonyl complexes to designed peptides. Dithiol and phosphine artificial amino acids have been generated by covalent modification of a lysine residue. Additionally, an FMOC-protected phosphine-serine derivative has been prepared that can be used in solid phase peptide synthesis. These methods have been used to incorporate metallocenters into water-exposed locations on α-helical and β-sheet peptides, but the catalytic properties were not modified from the small molecule analogues. In a second avenue of research, two new methods for immobilization of protein electrocatalysts at transparent surfaces have been developed. Proteins can be adsorbed to thin (10 nm) gold modified with an alkyl thiol layer, and the resulting submonolayer protein assembly investigated via both UV-vis spectroscopy and electrochemistry. Functional covalent modification of a silane layer on indium tin oxide could also be used as a biocompatible surface for adsorption of proteins.

15. SUBJECT TERMS

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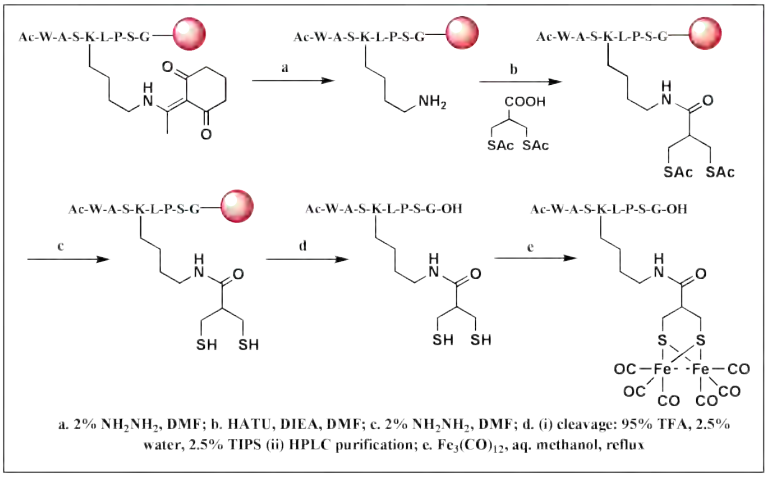
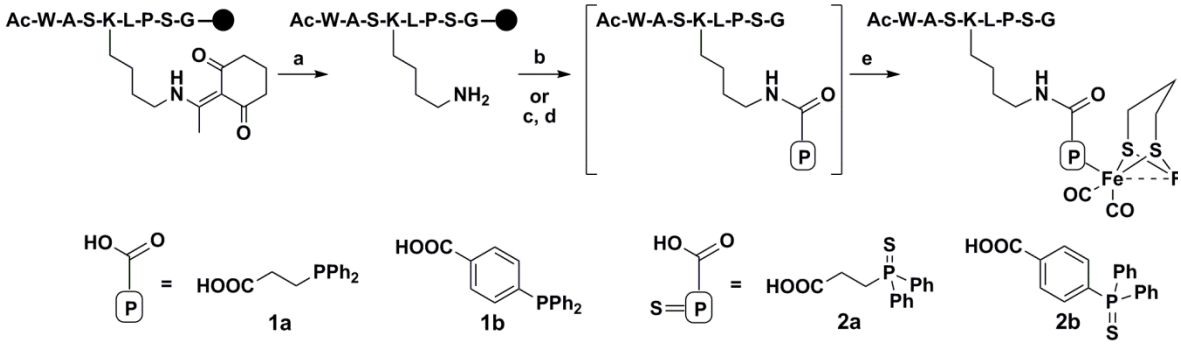


Scheme 1. Synthetic strategy for modification of a unique lysine with a dithiol functional unit and incorporation of an $Fe_2(CO)_6$ unit. The sphere represents the resin bead utilized for solid phase peptide synthesis.

derivative of lysine bearing a propandithiol unit (1)[1]. This dithiol unit precisely positions the two required sulfur atoms for the formation of a [(μ-SRS){$Fe(CO)_3$}$_2$] cluster on reaction with [$Fe_3(CO)_{12}$]. The resulting cluster can also be substituted with phosphine ligands and has spectroscopic properties nearly identical to those reported for the propanedithiol complex. This suggests that incorporation into a peptide alone does not radically alter the properties of the metallocenter. This work was published in the European Journal of Inorganic Chemistry (1).



a. 2% NH_2NH_2, DMF; b. (i) 1a, HATU, DIEA, DMF; (ii) cleavage: 95% TFA, 2.5% water, 2.5% TIPS (iii) HPLC purification; c. (i) 2a or 2
HATU, DIEA, DMF (ii) cleavage: 95% TFA, 2.5% water, 2.5% TIPS (iii) HPLC purificiation d. Ra-Ni, CH_3CN/MeOH; e. μ-$S_2Fe_2(CO)_6$(p
aq. CH_3CN, Me_3NO

Scheme 2: Synthetic strategy for modification of a unique lysine to generate phosphine-peptide and subsequent incorporation of diiron cluster via the phosphine ligand. The solid sphere represents the resin.

The phosphine-substituted derivatives of the diiron hexacarbonyl complexes are more active catalysts than the carbonyl parent molecules. Thus we have developed a second strategy shown in Scheme 2 to attach a phosphine ligand to an artificial peptide and synthesize the phosphine-metallocenter directly. In analogy to the lysine modification with a dithiol ligand, we have modified lysine with a diphenylphosphine unit. The key to this strategy is protecting the phosphorous before incorporation into the peptide to prevent formation a phosphine oxide. We have used sulfur-protected phosphine, and the sulfur can be removed by reduction with Raney nickel. To date, the deprotection has proceeded with low yield, and we are currently optimizing this step before submitting the manuscript for publication (4).

[1] Reference numbers refer to publication list in this report.

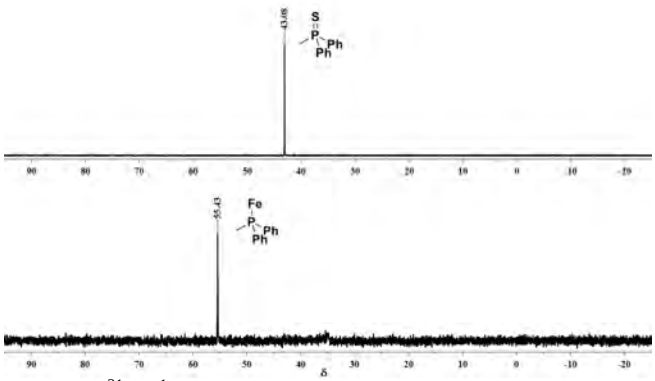
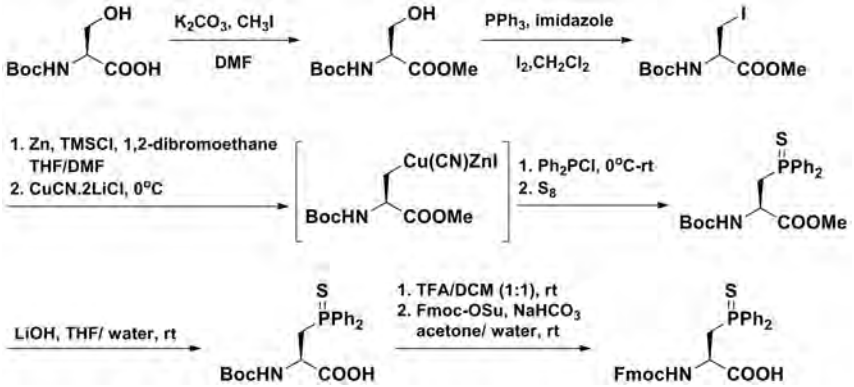


Figure 1: ^{31}P $\{^1H\}$ NMR spectra from (A) phosphine modified peptide, WASK{-$COC_2H_4P(S)Ph_2$}LPSG, with phosphine protected as diphenylphosphorothioyl and (B) (μ-pdt)$Fe_2(CO)_5$ cluster bound to peptide through phosphine coordination.

Modification of lysine produces a large tethered ligand when the side-chain of lysine itself is considered. There are many possibly rotamers for the side-chain, and this makes design of a peptide to encompass the metallocenter challenging. Thus we have also synthesized, diphenylphosphinoserine (pps) (Scheme 3), an artificial phosphine-amino acid that can be directly incorporated via solid phase peptide synthesis. This avenue will be pursued more aggressively as a means to introduce metallocenters at carefully placed positions in the interior of a protein after deprotection can be achieved with higher yield.

With respect to the impact of protein scaffold on metallocenter reactivity, to date we have incorporated diiron centers into α-helical and β-sheet secondary structures in largely solvent exposed sites. In these cases, the peptide has had little detectable impact on the metallocenter. To incorporate metallocenters into buried locations, we have considered both designed scaffolds and natural proteins. We have modified apo-cytochrome *C* with compound 1A in Scheme 2. The modified protein could then be used in reaction with [(μ-$SCH_2CH_2CH_2S$){$Fe(CO)_3$}$_2$] to form a [(μ-$SCH_2CH_2CH_2S$){$Fe(CO)_5(PPh_2R)$}] metallocenter in which R represents cytC. Unfortunately, the modification of the protein is non-specific and results in nonhomogeneous product that is difficult to characterize. It is nonetheless a tempting future direction for two reasons. First, the buried heme pocket provides an excellent, partially occluded location for alternative metallocenters. Second, cytC has been extremely amenable to electrocatalytic characterization. Thus artificial proteins derived from cytC are likely to form stable interactions with electrodes, an excellent starting point for development of electrocatalysts.



Scheme 3: Synthesis of artificial amino acid, diphenylphosphinoserine (Fmoc-pps-OH).

Development of novel mechanisms to interface proteins and electrodes

Since the long term goal of this work is to produce electrocatalysts. In parallel, we have investigated new methods to interface proteins with electrode surfaces. In particular, we have developed two methods that allow simultaneous electrochemical investigation and spectroscopic observation. Such techniques open doors to possible solar-powered current or fuel production applications.

We have shown that the proteins azurin and cytochrome *c* can be adsorbed to alkanethiol modified thin layer (10 nm) gold and observed spectroelectrochemically (Figure 2) without the addition of chemical mediators. This means that electron exchange is direct between the protein and the electrode. This is an exciting result since calculations based on solution extinction

coefficients suggested these UV-vis signals should not be detectable. However, we discovered that interaction of the gold surface plasmon with the proteins' electronic transitions causes a modest (between 8 and 100 times) surface enhancement making measurements possible. Furthermore, fast reversible electrochemical signals comparable to those seen on bulk gold electrodes were observed. This is significant since thin gold is a quasi-continuous substrate consisting of loosely connected islands of gold. This work has been favorably reviewed by Langmuir and is currently being revised for resubmission (2).

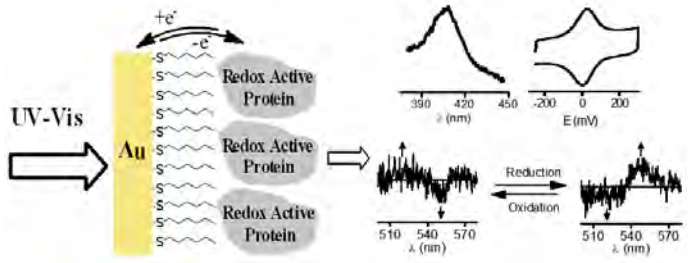


Figure 2: Schematic of adsorbed configuration of protein on thin gold for spectroelectrochemical investigation. UV-vis spectrum and cyclic voltammogram for adsorbed cytC are shown in the top row, and difference UV-vis spectra for cytC produced via oxidation/reduction at the electrode surface are shown in the bottom row.

Indium tin oxide (ITO) is widely used for inorganic applications such as solar cells but tends to denature proteins. Following literature methods, we have demonstrated that ITO can be functionalized with a silane layer to produce relatively dense films. By functionalizing the other end of the silane, we can create a protein-compatible interface. Using this methodology, we have successfully immobilized azurin on ITO, a remarkable result since all previous published attempts have resulted in denatured protein. This work is being reviewed for publication in Electrochemistry Communications (3).

As a direct result of my interactions with other researchers in the AFOSR program, I am currently preparing a review of hydrogenase electrocatalysis to appear as part of an edited book describing enzymatic fuel cells. This chapter will describe both applications of the naturally occurring enzymes and artificial electrocatalysts (5).

3. Personnel Supported:

Dr. Anne Katherine Jones
Mr. Nicholas Teodori
Mr. Souvik Roy

4. Publications:

Published:

1. S. Roy, S. Shinde, G. A. Hamilton, H. E. Hartnett, A. K. Jones. Artificial [FeFe] hydrogenase: On resin modification of an amino acid to anchor a diiron-hexacarbonyl cluster in a peptide framework. Eur. J. Inorg. Chem. 2011, 7, 1050-1055.

In revision or review:

2. I. Ashur, O. Schulz, C. McIntosh, I. Pinkas, R. Ros, A. K. Jones**.** Transparent gold as a platform for unmediated protein spectroelectrochemistry: investigation of cytochrome *c* and azurin. *In revision*. Langmuir.

3. I. Ashur, A. K. Jones*. Utilization of a functionalized silatrane to tether the redox protein azurin to indium tin oxide for direct electrochemistry. *In review*, Electrochemistry Communications.

In late stages of preparation*:*

4. S. Roy, A. K. Jones, (to be submitted 1/12) Synthetic [FeFe]-hydrogenases: Utilization of phosphine functionalize peptide ligands to construct (μ-$SCH_2CH_2CH_2S)Fe_2(CO)_5(PPh_2R)$ complexes. Dalton Transactions.

5. C. L. McIntosh, A. Dutta, P. Kwan, S. Roy, S. Yang, A. K. Jones, Bioelectrocatalysis of hydrogen oxidation and production. In *Enzymatic fuel cells: From fundamentals to applications*. Edited by H. Luckarift, G. Johnson and P. Attanasov. Note that this is an invited submission that arose out of interaction with the Air Force Laboratories. It is to be peer-reviewed with a deadline of Jan 31, 2012.

5. Presentations:

1. A. K. Jones, Bio-inspired catalysts for hydrogen oxidation and evolution. 5th Sante Fe Workshop on Materials for Energy Conversion (Topic: Bioinspired catalysts for oxygen reduction), NM, Nov. 2011.
2. A. K. Jones, Artificial Hydrogenases: Construction of peptide models for [FeFe]-hydrogenases, Main Group Chemistry Symposium, Southwest regional ACS meeting, Austin, TX, November 2011.
3. A. K. Jones, Engineering oxidoreductases: understanding the roles of residues outside the active site in controlling catalysis by hydrogenases, Boston University, October 2011.
4. A. K. Jones, Redox enzymes are electrocatalysts: exploration of natural and artificial hydrogenases, University of New Mexico, October 2011.
5. A. K. Jones, Artificial [FeFe] hydrogenase: modifications of amino acids to create ligands for binding diiron clusters, AFOSR Bioenergy Annual Review, Washington, D. C., USA, June 2011.
6. A. K. Jones, Exploring and Exploiting Redox Enzymes, Department of Chemistry Seminar Series, University of Nevada at Reno, 5 November 2010.
7. A. K. Jones. Artificial hydrogenases: Construction of peptide based models of hydrogenases. 9th International Hydrogenase Conference. Uppsala, Sweden. 30 June 2010.
8. A. K. Jones, Engineering Oxidoreductases: Design and Synthesis of Artificial Hydrogenases, Air Force Office of Scientific Research Annual Program Review, Arlington, VA, July 2010.
9. A. K. Jones, Design and Synthesis of artificial hydrogenases. Bioenergetics symposium of the Biophysical Society Meeting, San Francisco, CA, USA, 10 February 2010.
10. A. K. Jones**,** Engineering oxidoreductases: Design and Synthesis of Aritificial Hydrogenases, Air Force Office of Scientific Research Annual Program Review, Arlington, VA, Aug. 2009.
11. A. K. Jones, Exploring and Exploiting Redox Enzymes, Department of Civil, Environmental and Sustainable Engineering, Arizona State University, Tempe, AZ, USA, 24 Feb. 2009.
12. A. K. Jones, Exploring and Exploiting Redox Enzymes, Department of Chemistry Seminar Series, Regis College, Denver, CO, USA, 16 Feb 2009.
13. A. K. Jones, Artificial Redox Enzymes, Minisymposium on redox enzymes, University of Pennsylvania School of Medicine, Philadelphia, PA, USA, Nov 19, 2008.

14. A. K. Jones. Design and Synthesis of Artificial Hydrogenases. Fe-S Enzymes Gordon Research Conference, Colby-Sawyer College, New London, NH, June 2008.

6. Patent Disclosures: None.